Review

Clinical Cancer Research

Role of the RANK/RANKL Pathway in Multiple Myeloma

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Abstract

Receptor activator of nuclear factor-kappa B (RANK) and its ligand, RANKL, are expressed in a variety of tissues throughout the body; their primary role is in the regulation of bone remodeling and development of the immune system. Consistent with these functions, evidence exists for a role of RANK/RANKL in all stages of tumorigenesis, from cell proliferation and carcinogenesis to epithelial–mesenchymal transition to neoangiogenesis and intravasation to metastasis to bone resorption and tumor growth in bone. Results from current studies also point to a role of RANK/RANKL signaling in patients with multiple myeloma, who have increased serum levels of soluble RANKL and an imbalance in RANKL and osteoprotegerin. Current therapies for patients with multiple myeloma demonstrate that RANKL may be released by tumor cells or osteoprogenitor cells. This article will review currently available evidence supporting a role for RANK/RANKL signaling in tumorigenesis, with a focus on patients with multiple myeloma.

Introduction

Receptor activator of nuclear factor-kappa B (RANK) and its ligand, RANKL, are part of the tumor necrosis factor (TNF) superfamily. RANK and RANKL are expressed in a wide variety of tissues, such as kidney, liver, thymus, lung, and mammary tissue, but are most strongly expressed in bone, consistent with their primary role in regulating bone remodeling through osteoclast differentiation and survival (1). RANKL signaling also plays a role in the immune system through activation and survival of T cells (2). RANKL is considered to be one of the key factors at the crossroads of bone metabolism and immunity (3).

Multiple lines of preclinical and clinical evidence exist to support a role of RANK/RANKL in tumorigenesis of solid tumors, from initial stages of transformation to migration, metastasis, and bone resorption (2). Consequently, abrogation of RANK/RANKL signaling represents a target for cancer therapy at multiple stages. This article will review clinical and preclinical evidence for RANK/RANKL in the tumorigenesis of multiple myeloma, and current evidence for RANKL inhibition as a potential therapy for bone disease in patients with multiple myeloma. Reviews outlining the roles of other pathways in multiple myeloma have been recently published (4–7).

RANK/RANKL Structure and Function

RANK and its ligand, RANKL, are trimeric proteins involved in numerous processes in various tissues through triggering of multiple signaling cascades (Fig. 1; ref. 2). Osteoprotegerin (OPG) and other protagonists regulate the binding of RANKL or RANK, which are in turn regulated by other signaling factors. For example, OPG is controlled by numerous ligands such as TNF-related apoptosis-inducing ligand (TRAIL), von Willebrand factor (vWF), and glycosaminoglycans, which modulate the binding of OPG to RANKL (2). Many of the functions of RANK/RANKL are consistent with a potential role in tumorigenesis, which is consistent with evidence demonstrating its expression in numerous tumor types (2).

Bone remodeling

Bone remodeling, the primary function of RANKL signaling, is regulated by RANKL and OPG. This pathway is critical for the differentiation, activation, and survival of osteoclasts; accordingly, RANK or RANKL knockout mice have osteopetrosis and severe skeletal abnormalities due to their inability to remodel bone (8, 9). Conversely, mice deficient in OPG suffer early-onset osteoporosis and vascular calcification (10). RANKL is expressed primarily by osteocytes in addition to osteoblasts and other stromal cells; RANKL binding to its receptor on osteoclast precursors promotes their differentiation into bone-resorbing osteoclasts through activation of nuclear factor of activated T cells, which in turn activates dendritic cell–specific transmembrane protein (Fig. 2; ref. 11). OPG is released from osteoblasts to block osteoclast function and retain balance of bone formation and resorption: Simonet and colleagues demonstrated that recombinant OPG fused to the Fc protein inhibited osteoclast formation from spleen cells obtained from OPG-expressing and control mice (12). Within the bone marrow, osteoclasts are involved in the mobilization, maintenance, and regulation of hematopoietic stem cells, although they are not essential for this function (13, 14).

Immunity and inflammation

RANK signaling is important to immunity and inflammation; hematopoietic stem cells in the bone marrow are controlled by the immune system and work in concert with endocrine and neural systems to maintain bone homeostasis (3, 11). For example, RANK interfaces with RANKL, which is highly expressed on dendritic cells, to increase dendritic cell survival and enhance priming and activation of T cells; CD40 signaling has a redundant

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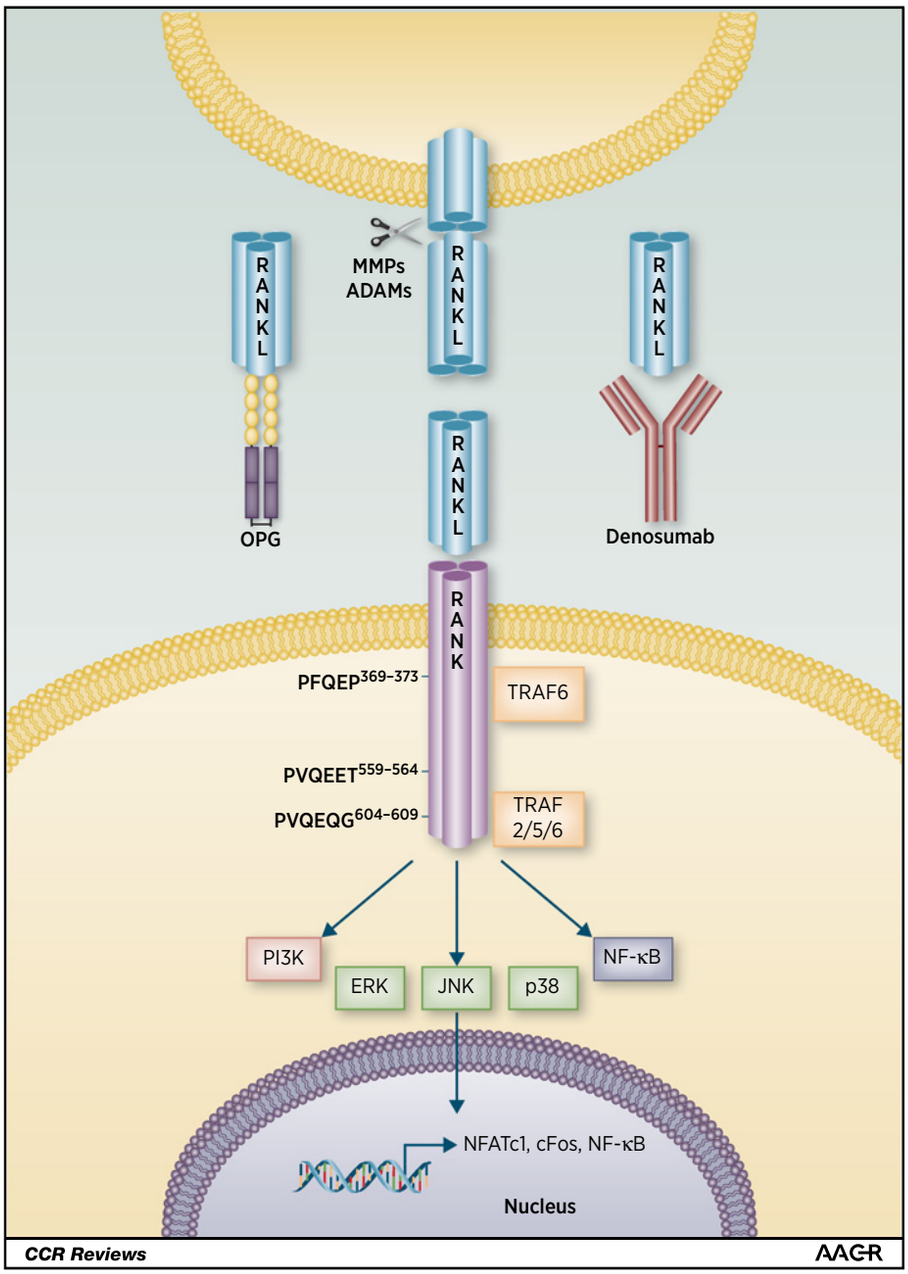

Figure 1.
Pathways activated by RANK/RANKL signaling and downstream targets; simplified schematic representation of the pathways activated after RANK and RANKL association. The natural decoy receptor for RANKL, OPG, and the clinical RANKL inhibitor, denosumab, are also represented. ADAMs, metalloprotease-disintegrins; ERK, extracellular signal-related kinases; JNK, cJun amino-terminal kinases; MMPs, matrix metalloproteinases; NF-κB, nuclear factor κB; NFATc1, nuclear factor of activated T cells, cytoplasmic 1; OPG, osteprotegerin; RANK, receptor activator of nuclear factor κB; RANKL, receptor activator of nuclear factor κB ligand; TRAF, TNF receptor-associated factor. Reprinted with permission from Gonzalez-Suarez E and Sanz-Moreno A. RANK as a therapeutic target in cancer. FEBS J 2016;283(11):2018–33, copyright John Wiley and Sons.

role in this RANK signaling function (15–17). RANK or RANKL knockout mice have altered B-cell development leading to decreased numbers of B cells (8, 9); however, those lacking RANK in B cells only have normal amounts of pro-, pre-, and immature B cells without alteration in basic B-cell function in humoral immunity (18). Thus, the effect of deleting RANK/RANKL on B-cell development is most likely secondary due to the lack of bone marrow cavities in these mice, suggesting that RANKL blockade does not affect normal B-cell physiology (18). RANKL signaling also plays a role in the establishment of central tolerance, which is mediated through dendritic cell control of $Foxp3^{+}$ regulatory T cells to regulate the response against self-antigens, food, and commensal flora (11, 17).

Cell growth and differentiation

In addition to bone remodeling, RANKL plays a role in epithelial cell growth and differentiation, including mammary gland differentiation (11). Mice deficient in RANK are not capable of lactation owing to lack of functional mammary gland formation during pregnancy (19). RANKL is also important to the formation of mammary glands by stimulating the proliferation of epithelial cells through nuclear factor kappa B (NF-κB); progesterone was important for this process through induction of RANKL (20). In a mouse mammary tumor virus model, direct action of RANKL was observed in tumorigenesis, which was accelerated by progesterone (21). RANKL also plays a role in hair renewal and epidermal growth in hair follicles,

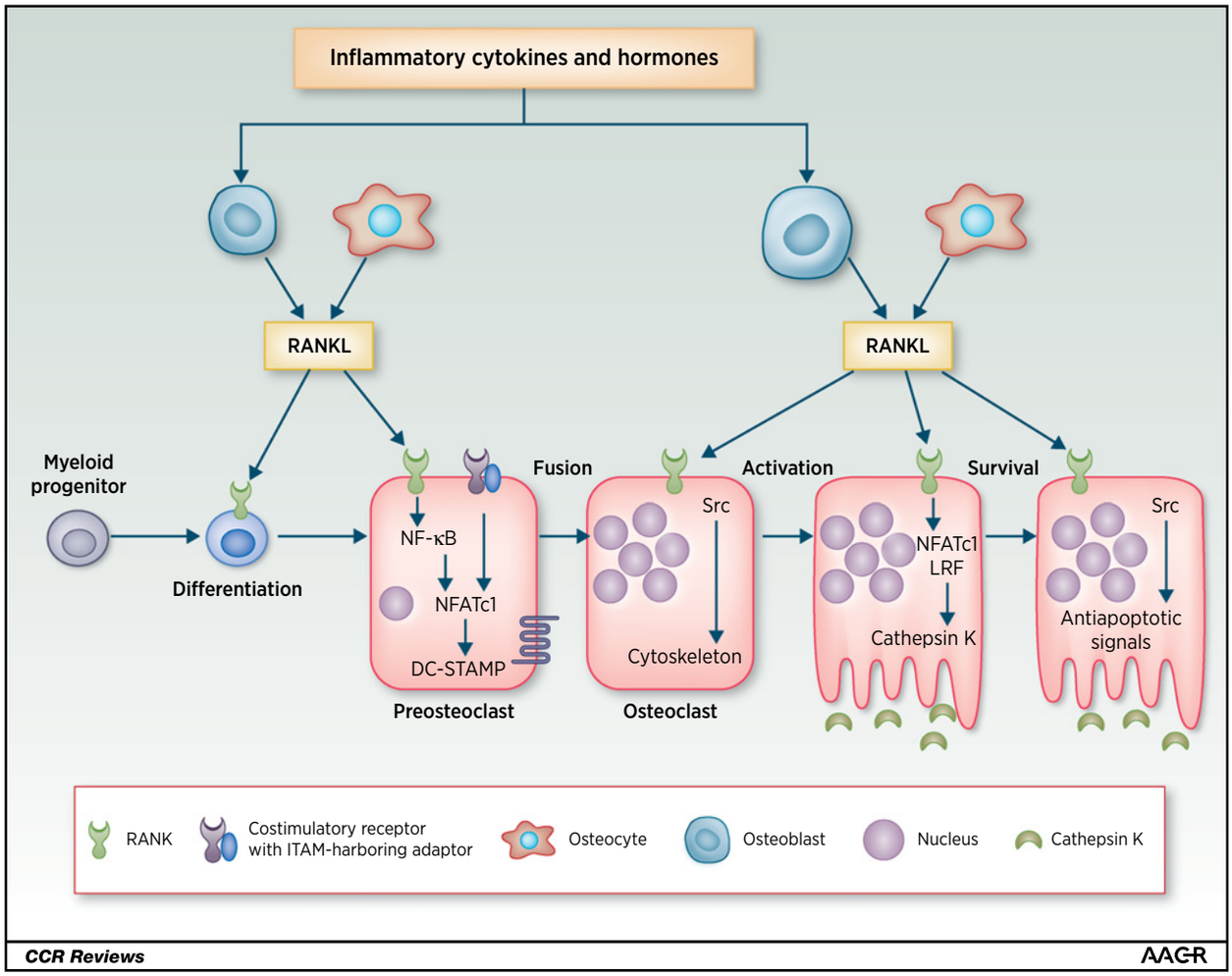

Figure 2.
Role of RANKL in differentiation, fusion, activation, and survival of osteoclasts. Myeloid progenitor cells progressively differentiate into osteoclast precursor cells, mononuclear preosteoclasts, and finally multinucleated osteoclasts, which are further activated to become mature bone-resorbing cells. After bone resorption, osteoclasts undergo apoptosis. RANKL regulates all of these processes. DC-STAMP, dendritic cell–specific transmembrane protein; ITAM, immunoreceptor tyrosine-based activation motif; LRF, leukemia/lymphoma-related factor; NF-κB, nuclear factor κB; NFATc1, nuclear factor of activated T cells, cytoplasmic 1; RANK, receptor activator of nuclear factor κB; RANKL, receptor activator of nuclear factor κB ligand; Src, proto-oncogene tyrosine-protein kinase. Reprinted with permission from Kukita A and Kukita T. Multifunctional properties of RANKL/RANK in cell differentiation, proliferation and metastasis. Future Oncol 2013;9(11): 1609–22, copyright Future Medicine Ltd.

stromal cell proliferation in the formation of secondary lymphoid tissues, differentiation of intestinal epithelial cells to microfold cells to form Peyer patches, and in the development and functions of medullary thymic epithelial cells (20).

RANK/RANKL in Multiple Myeloma

Multiple myeloma is a malignant proliferation of monoclonal plasma cells derived from post–germinal center B cells. The causes are numerous, with evidence for genetic and molecular alterations as well as microenvironmental factors (Fig. 3; ref. 22). Genetic factors include chromosome 1q21 amplification and chromosomal translocations resulting in deregulation of multiple myeloma SET domain (MMSET) and fibroblast growth factor-3. Other changes include activation of NRAS, KRAS, and dysregulation of MYC or TP-53 (22). Multiple myeloma is generally preceded by monoclonal gammopathy of undetermined significance (MGUS), characterized by the presence of a limited number of clonal plasma cells without any evidence of end organ damage (23). A series of genetic events and changes in the bone marrow microenvironment (induction of angiogenesis, suppression of cell-mediated immunity, and development of paracrine signaling loops involving IL6 and VEGF) occur during the progression of MGUS to multiple myeloma (23). Approximately 90% of patients with multiple myeloma develop bone lesions (24), which frequently lead to skeletal-related events (SRE). In a long-term, randomized, double-blind, placebo-controlled clinical trial of 392 patients with multiple myeloma, nearly 50% of patients assigned to placebo experienced an SRE over a 21-month period (25).

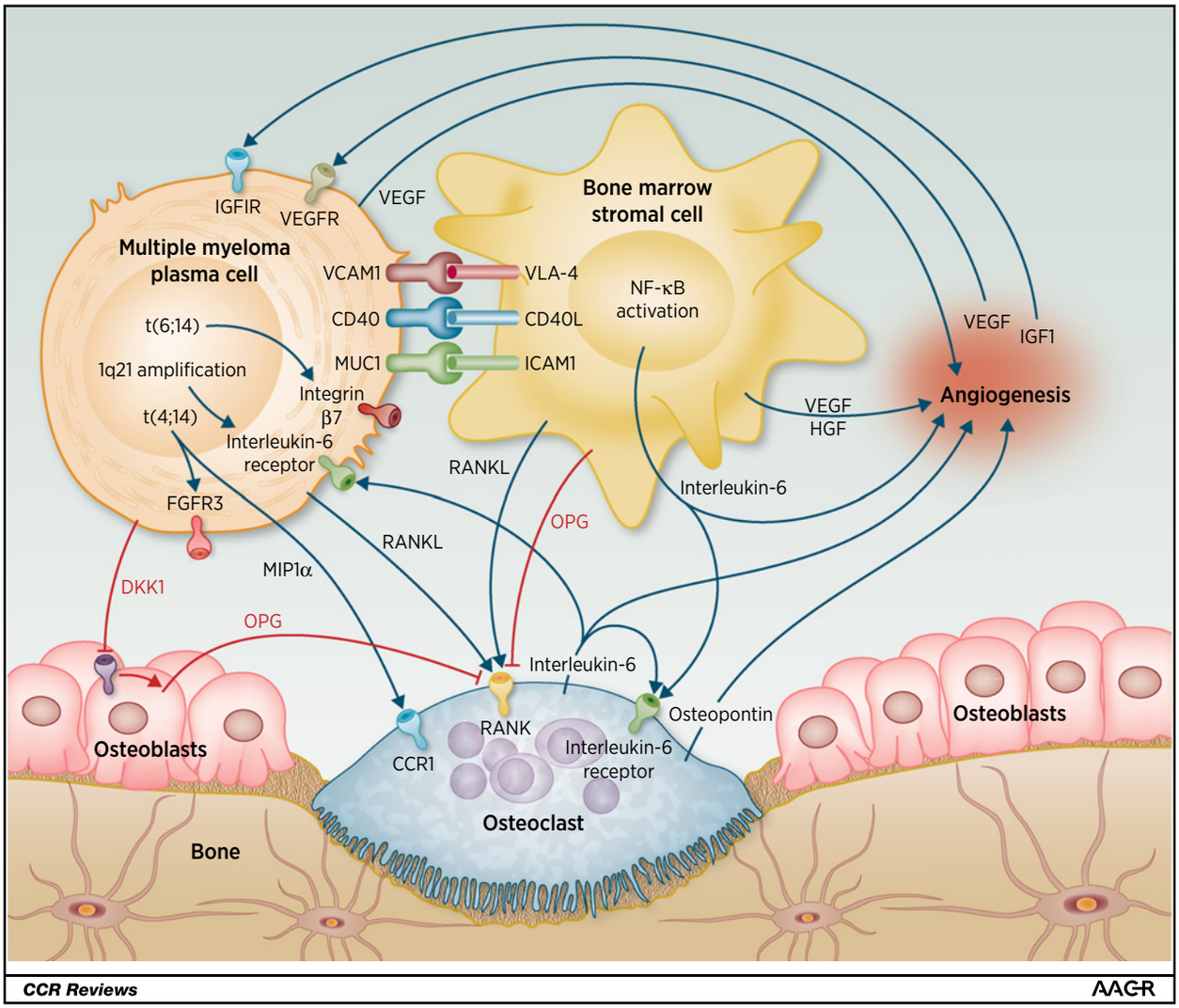

Figure 3.

Interaction between plasma cells and bone marrow in multiple myeloma. This interaction increases growth factor production, stimulating both plasma cells and angiogenesis. The increased osteoclast activity results from RANK/OPG imbalance due to enhanced RANKL production and reduced OPG production. Adhesion of plasma cells to stromal cells upregulates many cytokines with angiogenic activity (e.g., IL6 and VEGF); angiogenesis is also sustained by stromal cell–activated osteoclasts via osteopontin secretion. Chromosomal abnormalities can cause overproduction of receptors on myeloma cells. CCR, chemokine receptor 1; CD40L or CD40LG, CD40 ligand; FGFR3, fibroblast growth factor receptor 3; HGF, hepatocyte growth factor; ICAM1, intercellular adhesion molecule 1; IGF1, insulin-like growth factor 1; MIP1α, macrophage inflammatory protein 1 α; MUC1, cell-surface–associated mucin 1; NF-κB, nuclear factor κB; OPG, osteprotegerin; RANK, receptor activator of nuclear factor κB; RANKL, receptor activator of nuclear factor κB ligand; VCAM1, vascular-cell adhesion molecule 1; VLA-4, integrin alpha 4. From Palumbo A and Anderson K. Multiple Myeloma. N Engl J Med 2011;364(11):1046–60. Copyright 2011 Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society.

A hallmark of multiple myeloma is marked increase in osteoclast activity and resultant bone destruction (24). A vicious cycle exists in which cytokines released by multiple myeloma cells induce osteoclast activity and bone resorption, similar to the process for other tumor types. The increased bone resorption results in release of growth factors, which in turn stimulate growth of multiple myeloma cells, perpetuating the cycle (24). The RANK pathway is key to this process. In the 5T2MM mouse model of multiple myeloma, myeloma cells expressed RANKL, which directly promoted the formation of osteoclasts and osteolytic lesions. This was demonstrated by the ability of OPG-Fc to reduce the number of osteolytic lesions and increase bone mineral density compared with control (26). Similarly, in the humanized severe combined immunodeficiency (SCID-hu) mouse model of human multiple myeloma, the interaction of multiple myeloma cells with bone marrow stroma led to deregulation of the RANK–OPG cytokine axis, which is necessary for bone destruction and tumor progression; administration of RANK-Fc decreased tumor burden and production of multiple myeloma–promoting cytokines such as IL6 in the SCID-hu (27). These results demonstrate the interdependence of osteoclastogenesis and the survival of multiple myeloma cells (27).

Evidence for RANK/RANKL in multiple myeloma tumorigenesis

A wealth of evidence using cells from human and animal models implicates RANK/RANKL in multiple myeloma at various stages of the disease. mRNA and protein for soluble RANKL have been observed in samples from patients with multiple myeloma but not chronic lymphocytic leukemia, indicating a unique requirement for RANKL in multiple myeloma (28). In addition, treatment of the SCID/ARH-77 and SCID-hu-MM mice with either RANK-Fc or human immunoglobulin G directed at RANKL demonstrated that inhibition of RANKL delayed multiple myeloma–induced bone destruction and progression of the disease (29).

Studying patients with MGUS may also aid in establishing pathogenic mechanisms for multiple myeloma (Fig. 4; ref. 30). Indeed, patients with MGUS have RANK/OPG ratios that are greater than controls yet lower than patients with multiple myeloma (31), indicating early involvement of RANKL signaling and increased osteoclastogenesis in early stages of multiple myeloma. Experiments using the KMS12BM myeloma mouse model suggest that although the relative numbers of osteoclasts and osteoblasts remain stable until later stages of disease (8 weeks), rates of bone formation are reduced during the early stages of disease (3 weeks). Moreover, an even earlier effect (1 week) was observed in the form of increased cell cycling of osteoprogenitor cells by multiple myeloma cells (32). RANK gene expression was not upregulated

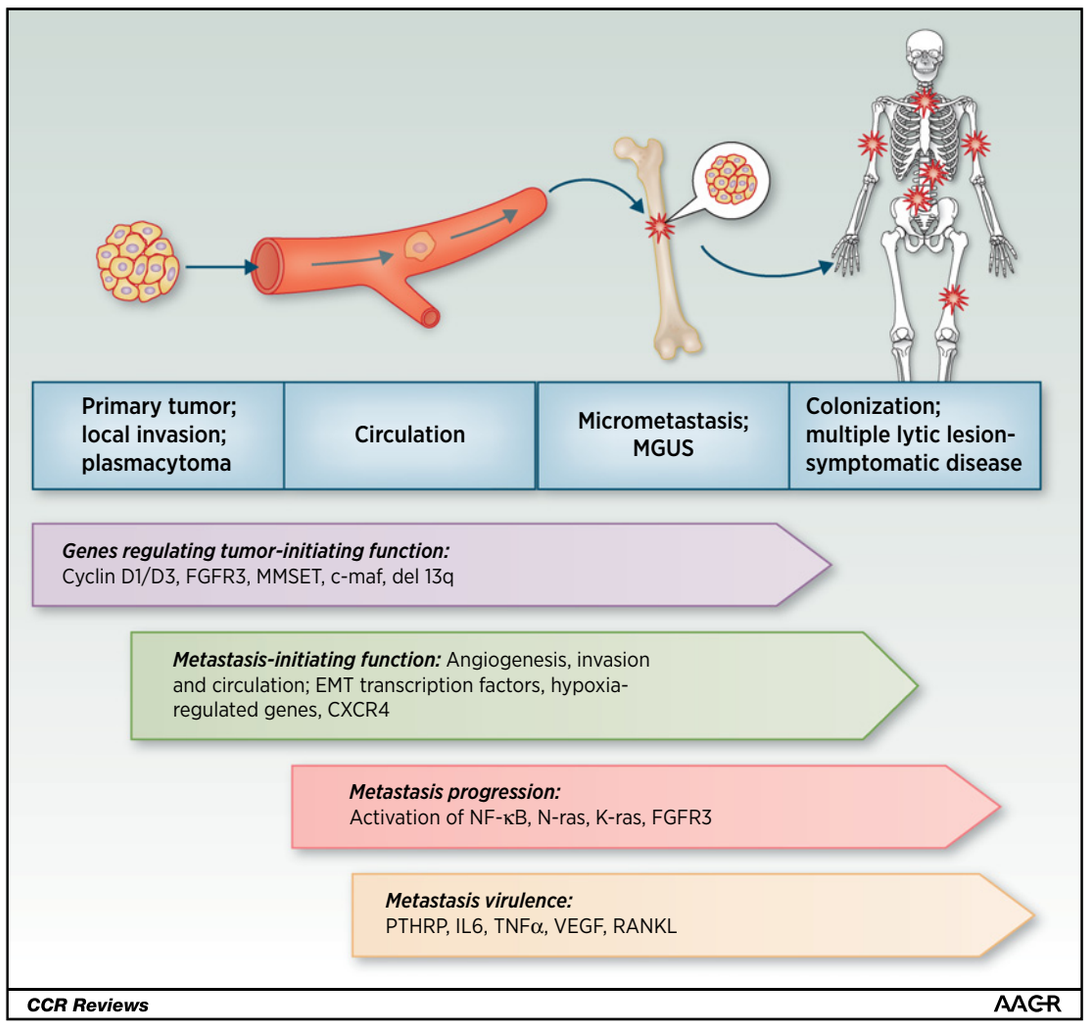

Figure 4.

Model of metastasis and dissemination in multiple myeloma. The initial site of tumor growth of clonal plasma cells is represented by a solitary plasmacytoma. Local plasmacytoma invasion occurs in most patients with multiple myeloma, which allows cells to egress into the peripheral circulation, followed by micrometastasis, and ultimately progression to macrometastasis or colonization after a long latency period. Genes representing tumor initiation and metastasis initiation, progression, and virulence are represented in the schematic. C-maf, avian musculoaponeurotic fibrosarcoma transcription factor; CXCR4, chemokine receptor type 4; del, delete; EMT, epithelial–mesenchymal transition; FGFR3, fibroblast growth factor receptor 3; K-ras, Kirsten rat sarcoma; MGUS, monoconal gammopathy of undetermined significance; MMSET, multiple myeloma SET domain; NF-κB, nuclear factor κB; N-ras, neuroblastoma rat sarcoma; PTHRP, parathyroid hormone-related protein; RANKL, receptor activator of nuclear factor κB ligand. Republished with permission of American Society of Hematology from Ghobrial IM. Myeloma as a model for the process of metastasis: implications for therapy. Blood 2012;120(1):20–3; permission conveyed through Copyright Clearance Center, Inc.

until 8 weeks, suggesting its involvement only in later stages of disease (32). Taken together, these results suggest that RANKL is progressively upregulated throughout the progression of multiple myeloma.

Bone marrow angiogenesis is an important process contributing to disease development and progression (33). Serum levels of RANKL and the RANKL/OPG ratio have been found to significantly correlate with markers of angiogenesis, including VEGF, in patients with multiple myeloma (34). In addition, in wild-type mice and using cultured human umbilical vein epithelial cells, RANKL was shown to promote vascular permeability and angiogenesis through stimulation of endothelial nitric oxide synthase; TNF receptor–associated factor 6 (TRAF6) and PI3K Akt were essential to this process (35).

Multiple myeloma bone disease

Multiple myeloma is a malignancy localized to the bone marrow arising from clonal proliferation of plasma cells. Typically at diagnosis, multiple myeloma manifests at multiple sites in the bone, suggesting spread, recirculation, and dissemination of tumor cells (30). Thus, dissemination of multiple myeloma to distant sites progresses through proliferation, epithelial–mesenchymal transition, angiogenesis and migration, and finally, metastasis (30, 36, 37).

Clinical evidence

Bone marrow biopsies from patients have clearly implicated expression of RANKL in multiple myeloma (38–41). Furthermore, serum levels of soluble RANKL correlate with osteoclast activation and bone resorption levels, as measured by tartrate-resistant acid phosphatase 5 (TRACP-5) and NTX. Similar to the observed preclinical data, an imbalance of RANKL/OPG exists in patients with multiple myeloma and this ratio may serve as a prognostic marker (42–44). Terpos and colleagues found that greater serum RANKL/OPG ratios were associated with shorter survival (43). At 60 months, the survival rate probability for patients with sRANKL/OPG <1 was 89% and for patients with a ratio of 1–3 was 32%. The survival rate probability for patients with a ratio >3 was <48 months. Levels of soluble RANKL also correlated with the extent of bone disease as examined by radiographic imaging (43).

Current and future treatments for multiple myeloma and their effects on RANK/RANKL

There are several therapeutic options for patients with multiple myeloma; each targets bone remodeling through different mechanisms.

Proteasome inhibitors. Proteasome inhibitors such as bortezomib, carfilzomib, and ixazomib act primarily on multiple components of osteoblast and osteoclast remodeling (38, 45). They also have an immunomodulatory effect through inhibition of the immunoproteasome and both direct and indirect effects on NK cells (46, 47). Direct effects include a proapoptotic effect on resting NK cells and inhibition of cytotoxicity of multiple myeloma cells toward NK cells; indirect effects include increased caspase 8 activity, resulting in increased susceptibility toward tumors, and downregulation of human leukocyte antigen class 1 on human multiple myeloma cells (46). Bortezomib is an intravenously or subcutaneously administered peptide boronic acid slowly reversible inhibitor of the β5 subunit of the proteasome (47). In patients with multiple myeloma, bortezomib decreased serum levels of RANKL and markers of bone resorption [TRACP-5b, C-telopeptide of collagen type 1 (CTX; ref. 48)]. Similarly, carfilzomib inhibits osteoclast differentiation and function at concentrations that are toxic to myeloma cells through a mechanism involving disrupted RANKL-induced NF-κB signaling (49). Ixazomib is a reversible peptide boronic acid that is orally administered (47). Both bortezomib and ixazomib are reported to prevent the degradation of Iκ-Bα, an inhibitor of NF-κB, leading to decreased NF-κB–mediated gene expression (50).

Immunomodulatory drugs. Thalidomide and its more potent analogue, lenalidomide, are used as immunomodulatory therapies in the treatment of patients with multiple myeloma (51). In patients with refractory/relapsed multiple myeloma, intermediate doses of thalidomide with dexamethasone led to signification reduction of the soluble RANKL/OPG ratio and markers of bone remodeling CTX and TRACP-5b (52). Similar results have been observed with lenalidomide, which moderately increased OPG expression while inhibiting RANKL expression, abrogated osteoclast differentiation and function, and inhibited secretion of multiple myeloma survival factors from osteoclasts and bone marrow stromal cells. However, it was unclear whether this was a direct effect of lenalidomide or an effect of reduction in tumor burden (53). Results of a randomized study help to clarify this issue: patients were treated with either dexamethasone plus lenalidomide or a combination of bortezomib and dexamethasone plus lenalidomide (54). Although lenalidomide plus dexamethasone reduced CTX, this effect was observed primarily in responders. Conversely, in patients who received bortezomib, dexamethasone plus lenalidomide, a reduction in dickkopf-1, RANKL/OPG, and CTX was observed, in addition to an increase in BALP and osteocalcin, demonstrating an effect on bone formation by bortezomib only (54). In a separate series of experiments, Bolzoni and colleagues demonstrated that the immunomodulatory drugs lenalidomide and pomalidomide prevented an imbalance in RANKL/OPG expression through inhibition of the interaction between myeloma and osteoprogenitor cells in coculture experiments (41).

Autologous stem cell transplantation. Autologous stem cell transplantation (ASCT) is used in combination with high-dose chemotherapy in eligible patients with multiple myeloma and offers a higher response rate and survival benefit over conventional chemotherapy (55). In a study of 51 patients who received high-dose chemotherapy followed by ASCT, markers of bone resorption were normalized (56). Levels of the bone resorption markers NTX, TRACP-5b, and soluble RANKL were significantly reduced compared with baseline beginning at 2 months post-ASCT and levels of bone formation markers osteocalcin and BALP increased after 9 and 11 months, respectively (56).

Bisphosphonates. Bisphosphonates are widely used in the treatment of patients with multiple myeloma and are recommended for patients with lytic bone disease or severe osteoporosis (57). Bisphosphonates currently recommended for use in patients with multiple myeloma are zoledronic acid, pamidronic acid, and clodronic acid (58). In an *in vitro* study using human osteoblasts, ibandronate and zoledronic acid strongly increased RANKL gene expression and promoted osteoblast proliferation (59). In contrast, the bisphosphonates pamidronic acid and ibandronate, which inhibited osteoclast activity, had no effect on tumor cell

growth (60). Importantly, ibandronate is ineffective to reduce SREs in patients with multiple myeloma (61). Finally, the Myeloma IX trial evaluated bisphosphonate therapy given before induction therapy (either cyclophosphamide–vincristine–doxorubicin–dexamethasone or cyclophosphamide–thalidomide–dexamethasone) in patients with newly diagnosed multiple myeloma. Zoledronic acid was shown to be superior to clodronic acid for both progression-free and overall survival, with a median overall survival of 52 months (62).

Immunotherapies. Daratumumab is a human mAb that targets CD38 on the surface of multiple myeloma cells to attract macrophages and NK cells to kill the multiple myeloma cells (7). Elotuzumab is a humanized mAb that targets the cell surface receptor SLAMF7 to elicit killing of multiple myeloma cells through stimulating NK cells when used in combination with lenalidomide and dexamethasone (7). The use of checkpoint inhibitors, ipilimumab and pembrolizumab, and chimeric antigen receptor T-cell therapy have demonstrated promising antitumor activity in early clinical trials in patients with multiple myeloma (7, 63–65), which are beyond the scope of this review.

Denosumab. Denosumab is a human mAb that inhibits the RANK ligand. In a previous randomized, controlled, phase III trial including 1,776 patients with bone metastases and solid tumors (excluding prostate and breast cancer) or multiple myeloma, treatment with denosumab was noninferior to zoledronic acid for the primary endpoint of time to first on-study SRE [HR = 0.84; 95% confidence interval (CI), 0.71–0.98; $P = 0.0007$ for noninferiority; ref. 66)]. Denosumab was not shown to be superior to zoledronic acid for time to first-and-subsequent SREs, and overall survival was similar between the two arms for the overall group (HR = 0.95; 95% CI, 0.83–1.08; $P = 0.43$; ref. 66). Although not powered for such an analysis, an unplanned *post hoc* analysis was conducted, which suggested that overall survival favored zoledronic acid over denosumab in 180 patients with multiple myeloma (HR = 2.26; 95% CI, 1.13–4.50; $P = 0.014$; refs. 66, 67). However, patients with multiple myeloma only accounted for 10% of the study population and because the primary endpoint was SREs, not survival or tumor response, the study design did not account for multiple myeloma–specific prognostic factors (67). Further exploration of the denosumab and zoledronic acid multiple myeloma subgroups revealed imbalances in baseline characteristics among the two treatment groups, and a higher rate of early withdrawals for the reasons of lost to follow-up and withdrawal of consent in the zoledronic acid group, suggesting that this result may have been confounded (67). Results from a recent phase III, randomized, double-blind trial (NCT01345019) comparing denosumab with zoledronic acid in 1,718 patients with multiple myeloma and bone disease demonstrated noninferiority of denosumab compared with zoledronic acid in delaying the time to first on-study SRE (HR = 0.98; 95% CI, 0.85–1.14; $P = 0.01$ for noninferiority; ref. 68). For the secondary and exploratory endpoints of overall survival and progression-free survival, respectively, HR (95% CI) were 0.90 (0.70–1.16; $P = 0.41$) and 0.82 (0.68–0.99; $P = 0.036$) for denosumab compared with zoledronic acid (68). Interestingly, the median progression-free survival was 10.7 months longer for denosumab (68). Because denosumab is a mAb, it is not dependent on the renal system for elimination and therefore can be used in patients with renal impairment (69). In the trial, the rate of renal adverse events was 17% in the zoledronic acid arm versus 10% in the denosumab arm (68). Hypocalcemia adverse events were reported in 12% of patients in the zoledronic acid arm and in 17% of patients in the denosumab arm. Because of the risk of osteonecrosis of the jaw (ONJ), it is recommended that patients beginning denosumab undergo a complete oral examination and that any invasive dental procedures are avoided while on denosumab therapy (70). In this phase III clinical trial, ONJ occurred in 4% of patients in the denosumab group and 3% of patients in the zoledronic acid group ($P = 0.147$; ref. 68). On the basis of these trial results, the FDA and the European Medicines Agency recently approved denosumab for the prevention of SREs in patients with multiple myeloma (71, 72).

Discussion and Conclusions

RANK/RANKL are central to bone remodeling and immune system development. This signaling pathway plays an important role in the development of bone disease in patients with multiple myeloma and several lines of evidence exist for a key role in the early stages of multiple myeloma. Therefore, inhibition of the RANK/RANKL pathway is an important therapeutic target in these patients. Denosumab, a RANKL inhibitor, was recently approved for patients with multiple myeloma. With the understanding of the role of RANK/RANKL in multiple myeloma, this should provide oncologists with the necessary knowledge to incorporate this new therapeutic option for patients with multiple myeloma.

Disclosure of Potential Conflicts of Interest

S. Bhatta holds ownership interest (including patents) in Amgen Inc. E. Terpos reports receiving speakers bureau honoraria from and is a consultant/advisory board member for Amgen Inc. No potential conflicts of interest were disclosed by the other author.

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References

1. Theoleyre S, Wittrant Y, Tat SK, Fortun Y, Redini F, Heymann D. The molecular triad OPG/RANK/RANKL: involvement in the orchestration of pathophysiological bone remodeling. Cytokine Growth Factor Rev 2004;15:457–75.
2. Renema N, Navet B, Heymann MF, Lezot F, Heymann D. RANK-RANKL signalling in cancer. Biosci Rep 2016;36. pii: e00366.
3. Takayanagi H. Osteoimmunology: shared mechanisms and crosstalk between the immune and bone systems. Nat Rev Immunol 2007;7:292–304.
4. Terpos E, Ntanasis-Stathopoulos I, Gavriatopoulou M, Dimopoulos MA. Pathogenesis of bone disease in multiple myeloma: from bench to bedside. Blood Cancer J 2018;8:7.

5. Terpos E, Christoulas D, Gavriatopoulou M, Dimopoulos MA. Mechanisms of bone destruction in multiple myeloma. Eur J Cancer Care 2017;26. doi: 10.1111/ecc.12761.
6. Gooding S, Edwards CM. New approaches to targeting the bone marrow microenvironment in multiple myeloma. Curr Opin Pharmacol 2016;28: 43–9.
7. Chim CS, Kumar SK, Orlowski RZ, Cook G, Richardson PG, Gertz MA, et al. Management of relapsed and refractory multiple myeloma: novel agents, antibodies, immunotherapies and beyond. Leukemia 2018;32:252–62.
8. Dougall WC, Glaccum M, Charrier K, Rohrbach K, Brasel K, De Smedt T, et al. RANK is essential for osteoclast and lymph node development. Genes Dev 1999;13:2412–24.
9. Kong YY, Yoshida H, Sarosi I, Tan HL, Timms E, Capparelli C, et al. OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. Nature 1999;397:315–23.
10. Bucay N, Sarosi I, Dunstan CR, Morony S, Tarpley J, Capparelli C, et al. Osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. Genes Dev 1998;12:1260–8.
11. Gonzalez-Suarez E, Sanz-Moreno A. RANK as a therapeutic target in cancer. FEBS J 2016;283:2018–33.
12. Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang MS, Luthy R, et al. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell 1997;89:309–19.
13. Tsirkinidis P, Terpos E, Boutsikas G, Papatheodorou A, Anargyrou K, Lalou E, et al. Bone metabolism markers and angiogenic cytokines as regulators of human hematopoietic stem cell mobilization. J Bone Miner Metab 2018;36:399–409.
14. Miyamoto K, Yoshida S, Kawasumi M, Hashimoto K, Kimura T, Sato Y, et al. Osteoclasts are dispensable for hematopoietic stem cell maintenance and mobilization. J Exp Med 2011;208:2175–81.
15. Anderson DM, Maraskovsky E, Billingsley WL, Dougall WC, Tometsko ME, Roux ER, et al. A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic-cell function. Nature 1997;390: 175–9.
16. Josien R, Wong BR, Li HL, Steinman RM, Choi Y. TRANCE, a TNF family member, is differentially expressed on T cell subsets and induces cytokine production in dendritic cells. J Immunol 1999;162:2562–8.
17. Akiyama T, Shinzawa M, Akiyama N. RANKL-RANK interaction in immune regulatory systems. World J Orthop 2012;3:142–50.
18. Perlot T, Penninger JM. Development and function of murine B cells lacking RANK. J Immunol 2012;188:1201–5.
19. Fata JE, Kong YY, Li J, Sasaki T, Irie-Sasaki J, Moorehead RA, et al. The osteoclast differentiation factor osteoprotegerin-ligand is essential for mammary gland development. Cell 2000;103:41–50.
20. Kukita A, Kukita T. Multifunctional properties of RANKL/RANK in cell differentiation, proliferation and metastasis. Future Oncol 2013;9: 1609–22.
21. Gonzalez-Suarez E, Jacob AP, Jones J, Miller R, Roudier-Meyer MP, Erwert R, et al. RANK ligand mediates progestin-induced mammary epithelial proliferation and carcinogenesis. Nature 2010;468:103–7.
22. Palumbo A, Anderson K. Multiple myeloma. N Engl J Med 2011;364: 1046–60.
23. Kyle RA, Rajkumar SV. Multiple myeloma. N Engl J Med 2004;351: 1860–73.
24. Roodman GD. Pathogenesis of myeloma bone disease. Leukemia 2009;23:435–41.
25. Berenson JR, Lichtenstein A, Porter L, Dimopoulos MA, Bordoni R, George S, et al. Long-term pamidronate treatment of advanced multiple myeloma patients reduces skeletal events. Myeloma Aredia Study Group. J Clin Oncol 1999;16:593–602.
26. Croucher PI, Shipman CM, Lippitt J, Perry M, Asosingh K, Hijzen A, et al. Osteoprotegerin inhibits the development of osteolytic bone disease in multiple myeloma. Blood 2001;98:3534–40.
27. Pearse RN, Sordillo EM, Yaccoby S, Wong BR, Liau DF, Colman N, et al. Multiple myeloma disrupts the TRANCE/osteoprotegerin cytokine axis to trigger bone destruction and promote tumor progression. Proc Natl Acad Sci U S A 2001;98:11581–6.
28. Schmiedel BJ, Scheible CA, Nuebling T, Kopp HG, Wirths S, Azuma M, et al. RANKL expression, function, and therapeutic targeting in multiple myeloma and chronic lymphocytic leukemia. Cancer Res 2013;73:683–94.
29. Sordillo EM, Pearse RN. RANK-Fc: a therapeutic antagonist for RANK-L in myeloma. Cancer 2003;97:802–12.
30. Ghobrial IM. Myeloma as a model for the process of metastasis: implications for therapy. Blood 2012;120:20–30.
31. Politou M, Terpos E, Anagnostopoulos A, Szydlo R, Laffan M, Layton M, et al. Role of receptor activator of nuclear factor-kappa B ligand (RANKL), osteoprotegerin and macrophage protein 1-alpha (MIP-1a) in monoclonal gammopathy of undetermined significance (MGUS). Br J Haematol 2004;126:686–9.
32. Kassen D, Lath D, Lach A, Evans H, Chantry A, Rabin N, et al. Myeloma impairs mature osteoblast function but causes early expansion of osteoprogenitors: temporal changes in bone physiology and gene expression in the KMS12BM model. Br J Haematol 2016;172:64–79.
33. Alexandrakis MG, Passam FJ, Ganotakis E, Dafnis E, Dambaki C, Konsolas J, et al. Bone marrow microvascular density and angiogenic growth factors in multiple myeloma. Clin Chem Lab Med 2004;42:1122–6.
34. Sfiridaki K, Pappa CA, Tsirakis G, Kanellou P, Kaparou M, Stratinaki M, et al. Angiogenesis-related cytokines, RANKL, and osteoprotegerin in multiple myeloma patients in relation to clinical features and response to treatment. Mediators Inflamm 2011;2011:867576.
35. Min JK, Cho YL, Choi JH, Kim Y, Kim JH, Yu YS, et al. Receptor activator of nuclear factor (NF)-kappaB ligand (RANKL) increases vascular permeability: impaired permeability and angiogenesis in eNOS-deficient mice. Blood 2007;109:1495–502.
36. Moschetta M, Kawano Y, Sacco A, Belotti A, Ribolla R, Chiarini M, et al. Bone marrow stroma and vascular contributions to myeloma bone homing. Curr Osteoporos Rep 2017;15:499–506.
37. Manier S, Sacco A, Leleu X, Ghobrial IM, Roccaro AM. Bone marrow microenvironment in multiple myeloma progression. J Biomed Biotechnol 2012;2012:157496.
38. Bingham N, Reale A, Spencer A. An evidence-based approach to myeloma bone disease. Curr Hematol Malig Rep 2017;12:109–18.
39. Roux S, Meignin V, Quillard J, Meduri G, Guiochon-Mantel A, Fermand JP, et al. RANK (receptor activator of nuclear factor-kappaB) and RANKL expression in multiple myeloma. Br J Haematol 2002; 117:86–92.
40. Qiang YW, Heuck CJ, Shaughnessy JD Jr, Barlogie B, Epstein J. Proteasome inhibitors and bone disease. Semin Hematol 2012;49:243–8.
41. Bolzoni M, Storti P, Bonomini S, Todoerti K, Guasco D, Toscani D, et al. Immunomodulatory drugs lenalidomide and pomalidomide inhibit multiple myeloma-induced osteoclast formation and the RANKL/OPG ratio in the myeloma microenvironment targeting the expression of adhesion molecules. Exp Hematol 2013;41:387–97.
42. Papadopoulou EC, Batzios SP, Dimitriadou M, Perifanis V, Garipidou V. Multiple myeloma and bone disease: pathogenesis and current therapeutic approaches. Hippokratia 2010;14:76–81.
43. Terpos E, Szydlo R, Apperley JF, Hatjiharissi E, Politou M, Meletis J, et al. Soluble receptor activator of nuclear factor kappaB ligand-osteoprotegerin ratio predicts survival in multiple myeloma: proposal for a novel prognostic index. Blood 2003;102:1064–9.
44. Spanoudakis E, Papoutselis M, Terpos E, Dimopoulos MA, Tsatalas C, Margaritis D, et al. Overexpression of RANKL by invariant NKT cells enriched in the bone marrow of patients with multiple myeloma. Blood Cancer J 2016;6:e500.
45. Accardi F, Toscani D, Bolzoni M, Dalla Palma B, Aversa F, Giuliani N. Mechanism of action of bortezomib and the new proteasome inhibitors on myeloma cells and the bone microenvironment: impact on myeloma-induced alterations of bone remodeling. Biomed Res Int 2015;2015: 172458.
46. Krieg S, Ullrich E. Novel immune modulators used in hematology: impact on NK cells. Front Immunol 2012;3:388.
47. Gandolfi S, Laubach JP, Hideshima T, Chauhan D, Anderson KC, Richardson PG. The proteasome and proteasome inhibitors in multiple myeloma. Cancer Metastasis Rev 2017;36:561–84.
48. Terpos E, Heath DJ, Rahemtulla A, Zervas K, Chantry A, Anagnostopoulos A, et al. Bortezomib reduces serum dickkopf-1 and receptor activator of nuclear factor-kappaB ligand concentrations and normalises indices of bone remodelling in patients with relapsed multiple myeloma. Br J Haematol 2006;135:688–92.
49. Hurchla MA, Garcia-Gomez A, Hornick MC, Ocio EM, Li A, Blanco JF, et al. The epoxyketone-based proteasome inhibitors carfilzomib and orally

bioavailable oprozomib have anti-resorptive and bone-anabolic activity in addition to anti-myeloma effects. Leukemia 2013;27:430–40.
50. Kupperman E, Lee EC, Cao Y, Bannerman B, Fitzgerald M, Berger A, et al. Evaluation of the proteasome inhibitor MLN9708 in preclinical models of human cancer. Cancer Res 2010;70:1970–80.
51. Moreau P, San Miguel J, Sonneveld P, Mateos MV, Zamagni E, Avet-Loiseau H, et al. Multiple myeloma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2017;28:iv52–iv61.
52. Terpos E, Mihou D, Szydlo R, Tsimirika K, Karkantaris C, Politou M, et al. The combination of intermediate doses of thalidomide with dexamethasone is an effective treatment for patients with refractory/relapsed multiple myeloma and normalizes abnormal bone remodeling, through the reduction of sRANKL/osteoprotegerin ratio. Leukemia 2005;19:1969–76.
53. Breitkreutz I, Raab MS, Vallet S, Hideshima T, Raje N, Mitsiades C, et al. Lenalidomide inhibits osteoclastogenesis, survival factors and bone-remodeling markers in multiple myeloma. Leukemia 2008;22:1925–32.
54. Terpos E, Christoulas D, Kastritis E, Katodritou E, Papatheodorou A, Pouli A, et al. The combination of lenalidomide and dexamethasone reduces bone resorption in responding patients with relapsed/refractory multiple myeloma but has no effect on bone formation: final results on 205 patients of the Greek myeloma study group. Am J Hematol 2014;89:34–40.
55. Kumar SK, Callander NS, Alsina M, Atanackovic D, Biermann JS, Chandler JC, et al. Multiple myeloma, version 3.2017, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw 2017;15:230–69.
56. Terpos E, Politou M, Szydlo R, Nadal E, Avery S, Olavarria E, et al. Autologous stem cell transplantation normalizes abnormal bone remodeling and sRANKL/osteoprotegerin ratio in patients with multiple myeloma. Leukemia 2004;18:1420–6.
57. Terpos E, Sezer O, Croucher PI, Garcia-Sanz R, Boccadoro M, San Miguel J, et al. The use of bisphosphonates in multiple myeloma: recommendations of an expert panel on behalf of the European Myeloma Network. Ann Oncol 2009;20:1303–17.
58. Terpos E, Morgan G, Dimopoulos MA, Drake MT, Lentzsch S, Raje N, et al. International Myeloma Working Group recommendations for the treatment of multiple myeloma-related bone disease. J Clin Oncol 2013;31:2347–57.
59. Koch FP, Merkel C, Ziebart T, Smeets R, Walter C, Al-Nawas B. Influence of bisphosphonates on the osteoblast RANKL and OPG gene expression in vitro. Clin Oral Investig 2012;16:79–86.
60. Yaccoby S, Pearse RN, Johnson CL, Barlogie B, Choi Y, Epstein J. Myeloma interacts with the bone marrow microenvironment to induce osteoclastogenesis and is dependent on osteoclast activity. Br J Haematol 2002;116:278–90.
61. Menssen HD, Sakalova A, Fontana A, Herrmann Z, Boewer C, Facon T, et al. Effects of long-term intravenous ibandronate therapy on skeletal-related events, survival, and bone resorption markers in patients with advanced multiple myeloma. J Clin Oncol 2002;20:2353–9.
62. Morgan GJ, Davies FE, Gregory WM, Bell SE, Szubert AJ, Cook G, et al. Long-term follow-up of MRC Myeloma IX trial: survival outcomes with bisphosphonate and thalidomide treatment. Clin Cancer Res 2013;19:6030–8.
63. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 2012;12:252–64.
64. Rosenblatt J, Avigan D. Targeting the PD-1/PD-L1 axis in multiple myeloma: a dream or a reality? Blood 2017;129:275–9.
65. Fan F, Zhao W, Liu J, He A, Chen Y, Cao X, et al. Durable remissions with BCMA-specific chimeric antigen receptor (CAR)-modified T cells in patients with refractory/relapsed multiple myeloma. J Clin Oncol 35:18s, 2017 (suppl; abstr LBA3001).
66. Henry DH, Costa L, Goldwasser F, Hirsh V, Hungria V, Prausova J, et al. Randomized, double-blind study of denosumab versus zoledronic acid in the treatment of bone metastases in patients with advanced cancer (excluding breast and prostate cancer) or multiple myeloma. J Clin Oncol 2011;29:1125–32.
67. Raje N, Vadhan-Raj S, Willenbacher W, Terpos E, Hungria V, Spencer A, et al. Evaluating results from the multiple myeloma patient subset treated with denosumab or zoledronic acid in a randomized phase 3 trial. Blood Cancer J 2016;6:e378.
68. Raje N, Terpos E, Willenbacher W, Shimizu K, Garcia-Sanz R, Durie B, et al. Denosumab versus zoledronic acid in bone disease treatment of newly diagnosed multiple myeloma: an international, double-blind, double-dummy, randomised, controlled, phase 3 study. Lancet Oncol 2018;19:370–81.
69. Block GA, Bone HG, Fang L, Lee E, Padhi D. A single-dose study of denosumab in patients with various degrees of renal impairment. J Bone Miner Res 2012;27:1471–9.
70. Ruggiero SL, Dodson TB, Fantasia J, Goodday R, Aghaloo T, Mehrotra B, et al. American Association of Oral and Maxillofacial Surgeons position paper on medication-related osteonecrosis of the jaw–2014 update. J Oral Maxillofac Surg 2014;72:1938–56.
71. OncLive. FDA approves denosumab for multiple myeloma; 2018. Available from: http://www.onclive.com/web-exclusives/fda-approves-denosumab-for-multiple-myeloma.
72. European Medicines Agency. Xgeva (denosumab). Summary of product characteristics; 2018. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/002173/WC500110381.pdf.